

# Fungal Extracts from Endophytes for Synthesis of Metal Nanoparticles



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## Abstract

Using fungal extracts as bio-reducing agents is considered an attractive approach, as they contain bioactive compounds (e.g., enzymes, proteins), which could serve as reducing and capping agents in synthesizing metal nanoparticles (mNPs). Biological synthesis coats the mNPs with biocompatible and biologically active compounds, which makes them more stable and enhances their biological potential. Biogenic fabrication of mNPs has been established using extracts from fungi. Nevertheless, the use of extracts from a specific group, "endophytes," has not been fully explored. Endophytic fungi are attractive sources due to the extracellular enzymes they produce and the bioactive compounds in the extract, which are said to be like the bioactive compounds of the host plant. The use of natural extracts from endophytes is a renewable resource, which is also environmentally friendly. This chapter describes the biological synthesis of mNPs using extracts from fungal endophytes, the possible synthesis mechanism involved, and the types of synthesized mNPs and their applications.

**Keywords:** Biogenic synthesis; Bio-reducing agents; Endophytic fungi; Fungal extracts; Environmentally friendly; Metal nanoparticles

**Abbreviations:** SDR: Spinning Disc Reactor; Mnps: Metal Nanoparticle; TEM: Transmission Electron Microscopic Micrographs

## Introduction

Nanotechnology is an integrative field of science, focusing on the synthesis of materials at the nanoscale level. The synthesized nanomaterials or nanoparticles are materials or particles with extremely small dimensions (1-100nm) and exceptional characteristics. They have a larger surface area to volume ratio [1]. Nanoparticles are typically synthesized via chemical and physical methods. The chemical methods include chemical reduction, sol-gel, and hydrothermal synthesis of mNPs [2,3]. Physical methods include applications of lithography, laser ablation, and high-energy irradiation [4,5]. Nonetheless, the involvement of high temperature and pressure requirements, and various toxic

and highly reactive chemicals for the respective physical and chemical methods is undesirable as it is often associated with potential health (carcinogenicity) and environmental hazards. All these toxicity concerns limit the synthesis of nanoparticles via conventional methods [6].

In recent years, biogenic synthesis has grown increasingly important as one of the favorable alternatives to conventional synthesis. Biogenic synthesis of mNPs utilizes plants, microorganisms, biopolymers, and enzymes, among others. The synthesis of nanoparticles using renewable biological entities offers a simple, safe, clean, and environmentally friendly method

to synthesize nanoparticles [7]. This approach addresses toxicity-related problems arising from conventional synthesis approaches [8]. Plants are one of the most extensively studied for the biological synthesis of nanoparticles [9]. Nevertheless, in recent years microbial extracts have become increasingly significant. Microbial-mediated synthesis of mNPs can be achieved due to the presence of biological molecules (i.e. proteins, sugars, and reducing enzymes) in bacteria, algae, fungi, and yeast [10]. Microbial extracts are diverse in their chemical profile, thus are suitable as bio-reducing and capping agents to synthesize mNPs. These bioactive compounds are also present in fungal endophytes.

Fungal endophytes are microorganisms that inhabit plant tissues without causing disease symptoms. They were first discovered in grasses, where endophytic fungi produced alkaloids that were toxic against the herbivores [11]. Endophytes provide resistance against pests and pathogens and hence can be used as biocontrol agents [12]. Fungal endophytes are a potential source of numerous bioactive compounds and secondary metabolites of therapeutic value. In recent years, fungal endophytes have been investigated to produce naturally occurring bioactive compounds and as biological control agents [13]. Fungal endophytes produce extracellular enzymes and proteins, which reduce metal ions into nanoparticles during the synthesis of mNPs.

### Metal nanoparticles

Metal nanoparticles (mNPs) are entities sized between 1-100nm and composed of pure metals or metal compounds (e.g., sulfides, oxides, chlorides, and fluorides). Metal nanoparticles exhibit many unique characteristics including surface plasmon resonance and optoelectrical properties. Their sizes are uniform and have a large surface area to volume ratio. As such, they are useful in applications in biological and biomedical sciences. Other than optoelectrical properties, mNPs also show a diverse therapeutic potential as antimicrobial, antiangiogenic, and anticancer agents [14]. Metal nanoparticles are categorized into four types depending on their dimensions. This includes mNPs with zero-dimensional, one dimension, two dimensions, and three dimensions. Zero-dimensional nanoparticles are the most common form of mNPs that have all dimensions in the nanoscale (e.g., nanoparticles). One-dimensional nanoparticle has two dimensions at the nanoscale and one dimension is outside the nanoscale, they include nanorods and nanowires. In two-dimensional nanoparticles, only one dimension is at the nanoscale while the other two dimensions are not in the nanoscale. They are highly functional in terms of chemical, electrical, and optical properties, they include nanofilms and nanolayers. Three-dimensional nanoparticles have all dimensions outside the nanoscale, this group contains powders of nanoparticles and accumulations of nanowires [15].

### Synthesis of metal nanoparticles

Nanoparticles are synthesized via two main approaches. The first is where the bulk material is reduced to nanoparticles. In

this method, the synthesis of nanoparticles is initiated from bulk material, during which the solid-state processing (the process where the bulk material is subjected to mechanical forces), converts the bulk material into smaller particles through different physical methods (i.e. grinding, crushing). Nanoparticles prepared from these methods are not uniform in shape or size, therefore these approaches are considered less effective. The other drawback that is associated with this approach is the flaws in the surface morphology of the synthesized nanoparticles. These surface structure-related imperfections significantly affect the physical and chemical properties of nanomaterials. The other approach is where nanoparticles are synthesized by the assemblage of atoms, molecules, or clusters. This approach is much more reliable with nanoparticles synthesized of the same shape and size. It involves the chemical fabrication of nanoparticles, where the reaction conditions are controlled and optimized carefully to obtain the desired yield. The following sections discuss the conventional and biological methods for the synthesis of mNPs.

### Conventional methods of metal nanoparticle synthesis

Conventional techniques for the synthesis of mNPs include various physical and chemical approaches. The physical approaches include mechanical milling, nanolithography, laser ablation, thermal decomposition, and electrospinning, while the chemical methods include pyrolysis, sol-gel, hydrothermal and solvothermal. All methods can produce mNPs of acceptable quality, although some techniques have certain limitations. Among the various physical techniques, mechanical milling is the most well-known method. It is used for the crushing and post-annealing of nanoparticles during the synthesis. There are some factors (fractures, deformations, and welding), which strongly influence the shape and size of the nanoparticles produced [16]. The method is low-cost, with a higher yield of mNPs. Mechanical milling can lead to the production of powdered mNPs that tend to agglomerate easily, making it challenging to control their size and shape [17]. The other physical technique is nanolithography. Nanolithography is used to synthesize nanoparticles of 1-100nm sizes. This is performed by pressing and heating a thin layer between an imaged template and a solid substrate, where the film attaches to the substrate upon heating. Nanolithography allows the production of clusters of nanoparticles with the same size and shape, from a single nanoparticle. This method has a high throughput and is inexpensive compared to other lithography techniques [18]. Laser ablation is another method that is used to synthesize mNPs using different solvents. This method involves the condensation of a plasma plume that produces nanoparticles via laser ablation of bulk material in a solvent.

It is considered a biological synthesis method, as it synthesizes mNPs using water and organic solvents without the involvement of stabilizing chemicals [19,20]. Electrospinning is another favorable method, used to synthesize nanofibers. In this method, the mNPs synthesis is carried out in a spinning disc reactor (SDR), where the rotating disc is present inside the chamber. SDR is filled

with some inert gases such as nitrogen, and the oxygen is removed from the reactor to avoid chemical reactions. Due to the rotation of the spinning disc, atoms and molecules are fused and precipitates are formed which are recovered and dried. Electrospinning can produce nanofibers in the range of 1-10 nm, although this advantage is countered by the different toxic solvents used [21].

For chemical methods, pyrolysis is one of the most frequently used techniques, especially for the synthesis of mNPs at an industrial scale. In pyrolysis, the precursor is added into the furnace via a hole at high pressure for decomposition. Metal nanoparticles are then separated and recovered. It is an efficient, simple, and economical approach that enables the high throughput production of nanoparticles [22]. The sole gel is another important chemical method that is used to synthesize mNPs. The method in sole gel comprises three main steps: hydrolysis, condensation, and drying. The hydrolysis of precursor metal yields the metal hydroxides, which immediately undergo condensation to form gels. The obtained gel is then dried and mNPs are recovered via centrifugation, filtration, and sedimentation. The sole gel method involves only low temperatures (25-100°C) for the synthesis of nanoparticles. The sol-gel method, however, demands the use of costly chemicals, and very fine particles are produced [2,23]. Solvothermal is the other chemical method that allows for the synthesis of nanoparticles. This is performed in a closed stainless-steel container where reactants are heated at high temperatures in the presence of organic solvent. Heating causes chemical alteration and a series of chemical transformations that lead to the synthesis of nanoparticles [24]. In addition, hydrothermal synthesis of nanoparticles also shares the same synthesis principle, but water is used instead of a solvent, therefore it is referred to as the hydrothermal synthesis approach. This method is commonly used for the synthesis of mNPs with extremely small sizes through

optimization of process parameters (i.e., temperature and pressure), to control the size and shape of nanoparticles produced [3].

### Biological methods for synthesis of metal nanoparticles

Metal nanoparticles can be synthesized using biological materials, involving the use of plants, algae, bacteria, and fungi. The biological extracts produced are rich in biological constituents, which are effective in the synthesis of mNPs. Biological methods are generally safe and environmentally friendly [1]. The biological synthesis of mNPs addresses toxicity concerns associated with other physicochemical methods.

The biological method is another favorable method that is used to synthesize mNPs. In this method, biological extract is used as a bio-reducing agent which causes the reduction of metal ions. The biological extract is treated with precursor metal salt solution, the reaction mixture is incubated at room temperature under ambient conditions. There are some reaction parameters including concentrations of biological extract and metal solution, pH of the reaction mixture, temperature, agitation, and incubation time, which greatly affect the shape and size of biosynthesized mNPs [25]. The biological synthesis of mNPs is comprised of three main steps bio-reduction, growth or synthesis, and stabilization of biosynthesized mNPs. In the first step of the synthesis, the bio-reduction of metal ions is carried out using a biological extract that acts as a reducing agent and leads to the reduction of metal ions into mNPs. In the following second step reduced metal particles are mechanically adjoined and lead to the formation of mNPs. In the final third step synthesized nanoparticles attain their stability, and this step determines the shape of synthesized mNPs (Figure 1). The following sections discuss the conventional and biological methods for the synthesis of mNPs (Table 1).

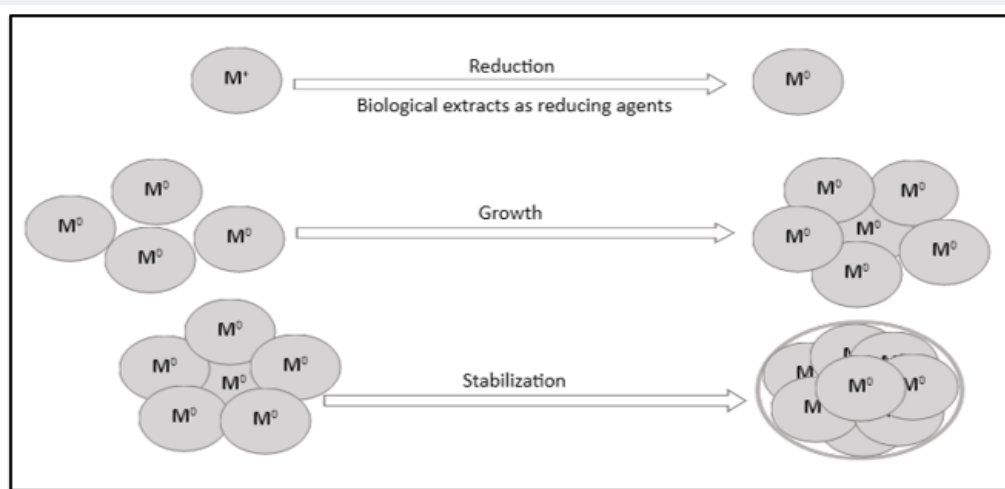


Figure 1: Mechanism of metal nanoparticles synthesis using biological extracts as reducing agents.

Plants are the most suitable and attractive biological resources which are used for the biogenic synthesis of mNPs. They are the natural bio-factories that are capable of synthesizing mNPs, as they produce a wide range of biologically active phytochemicals. Various biologically active compounds of plant origin including alkaloids, phenols, proteins, sugars, polyphenols, and coenzymes are acknowledged as excellent bio-reducing agents which also stabilize the mNPs [26]. In the green approach of mNPs synthesis plant parts, whole plants, or plant extracts are used which is a positive aspect of this method. Furthermore, plant-mediated biological synthesis of mNPs is a simple, rapid, easy, and economical approach. It is also considered an environmentally benign approach as it limits the use and production of various lethal chemicals which are a major part of chemical synthesis [27]. The first account on the green synthesis of nanoparticles using plants was reported by Gardea-Torres Dey, Gomez, Peralta-Video, Parsons, Troiani, and Jose-Yakama [28], since then numerous reports also have been published on the biological synthesis of mNPs using plants and plant-based products.

The other frequently used and important biological entities are bacteria. They have been reported for their metal nanoparticle synthesis potential, intracellular and extracellular synthesis of mNPs can be achieved using bacteria [29]. The common bacterial species that have been used to synthesize mNPs are *Escherichia coli*, *Pseudomonas sp.*, *Klebsiella pneumonia*, *Bacillus sp.*, *Lactobacillus sp.*, *Actinobacteria sp.*, *Corynebacterium sp.*, *Pseudomonas sp.* [30]. Biosynthesis of AuNPs using *Pseudomonas stutzeri* was carried out, in this process NADH-dependent reductase enzyme was involved for the bio-reduction of gold ions into AuNPs [31]. In another study, the extracellular fabrication of AuNPs was investigated from *Pseudomonas aeruginosa* [32].

Fungi have an excellent ability to produce mNPs of different chemical compositions, and it is a unique property of some fungal species. Biosynthesis of mNPs using fungal extracts is reported by several researchers, *Fusarium sp.*, *Aspergillus sp.*, and *Penicillium sp.*, are commonly used for the green synthesis of mNPs [33, 34]. They produce greater amounts of enzymes and protein which are responsible for the high yields of nanoparticles using fungal extracts [35]. The size and shape morphology of fungus-mediated mNPs mainly depend on the culture conditions, which strongly affect the mNPs fabrication. Extracellular synthesis of AuNPs was investigated under stationary conditions using *Trichothecium sp.* Continuous agitation of fungal mycelium caused the intracellular synthesis of AuNPs. Results indicated that stationary conditions promote the release of proteins and enzymes that cause extracellular synthesis, while agitation prevents the release of these enzymes hence intracellular synthesis occurs [36]. Algae are a group of eukaryotic aquatic organisms, which are used for the biogenic synthesis of mNPs. They have an excellent ability to accumulate heavy metal ions, though they can also be used as a potential biological source to synthesize nanoparticles. Gold nanoplates were fabricated at room temperature, using extract from *Chlorella vulgaris*. Results demonstrated the role of

extracting proteins which were involved in the reduction of gold ions into nanomaterials [37]. Similarly, the extracellular and intracellular synthesis of AuNPs was established using extracts from *Sargassum wightii*, *Kappaphycus alvarezii*, and *Tetraselmis kochinensis* [38-40].

### Biological synthesis of metal nanoparticles using plants

Plants and plant-based materials can be used for the biogenic fabrication of mNPs [45]. Plants are known as 'chemical factories', contain various compounds of biological potential, and are hence widely exploited for the synthesis of mNPs. Biosynthesis of mNPs using plant extracts is considered one of the most suitable and safer approaches, where higher yields of nanoparticles can be obtained using natural extracts that act as green catalysts and cause the reduction of metal ions [46]. Some of the most important phytochemicals which have a miraculous bio-reducing power include terpenoids, flavonoids, amides, sugars, carboxylic acids, aldehydes, and ketones. The chemical nature and diversity of phytochemicals in plants strongly affect the size and structural morphology of the synthesized nanoparticles, because each part of the plant has different concentrations of these phytochemicals [47].

The biological synthesis of mNPs has been widely studied by several researchers (Table 17.2). Biogenic synthesis of silver nanoparticles using aqueous fruit extract of *Cucumis sativus* was studied, and the resulting nanoparticles were characterized and tested for their antibacterial potential against *Staphylococcus aureus*, *Klebsiella pneumonia*, and *Escherichia coli*. Biosynthesized silver nanoparticles showed significant results [48]. In another study, mulberry leaf extract was used to synthesize silver nanoparticles, and bio-fabricated nanoparticles were found to be effective when investigated for their bactericidal potential [49]. Bio-fabrication of CuNPs was established using extracts from *Euphorbia nivulia* and *Syzygium aromaticum* [50,51]. Likewise, the aqueous leaf extract of *Ocimum sanctum* was used for the green synthesis of CuNPs, and the synthesis of nanoparticles was confirmed through different characterization techniques [52].

Biogenic synthesis of AuNPs using the aqueous extracts of *Malva crisp* and *Cucurbita pepo* was studied, resultant nanoparticles were characterized and assessed for their antimicrobial potential, and green fabricated AuNPs exhibited significant antibacterial activity against food spoilage bacteria [53]. Similarly, biological extracts of *Punica granatum* and *Cassia auriculata* were also investigated for the fabrication of AuNPs, and their anticancer activity was evaluated against different cancer cell lines [54,55]. Biogenic green synthesis of FeNPs was carried out using six plants, the synthesized green nanoparticles were characterized [56]. The fabrication of iron nanoparticles was also investigated using *Gardenia jasminoides*, *Dodonaea viscosa*, and *Lawsonia inermis* leaf extracts. Scanning electron microscopy analysis showed the shape and size of FeNPs that were observed as circular, and the average diameter was calculated as 27nm. Resulted nanoparticles also exhibited excellent antibacterial activity against all the assessed

bacteria [57,58].

The synthesis of palladium nanoparticles was also reported using aqueous extracts from *Annona squamosa*, *Diopyros kaki*, and *Curcuma longa* [59-61]. The biological synthesis of platinum nanoparticles was carried out using *Anacardium occidentale* leaf extract, where the reduction of metal ions was caused by the polyols, and proteins were found to be responsible for the stabilization of nanoparticles [62]. Selenium nanoparticles were produced using seeds extracted from fenugreek, the bio-fabricated nanoparticles were also assessed for their anticancer activity, where they showed significant results [63]. Similarly, biosynthesis of titanium dioxide nanoparticles has also been reported, using extracts from *Annona squamosa* and *Catharanthus roseus* [64-81].

### Biological synthesis of metal nanoparticles using microorganisms

Microorganisms are essential biological entities that play a crucial role in various ecological processes on Earth. Key classes of microbes include bacteria, fungi, algae, and viruses. The biological synthesis of mNPs using microbial extracts is gaining attention, as these organisms are viewed as natural nano-factories for fabricating mNPs. Previous studies have shown that both unicellular and multicellular microorganisms possess significant potential for producing metal nanomaterials. This biosynthesis occurs through a bottom-up approach, where mNPs are formed

via biological molecules such as proteins, sugars, and enzymes of microbial origin [82]. Each microorganism interacts differently with specific metal ions, and factors such as temperature and pH can influence the shape and size of the resulting nanoparticles. Therefore, further research is essential to elucidate the exact mechanisms behind the microbial-mediated synthesis of mNPs [83,84].

The mechanism of mNPs synthesis using microorganisms is somehow like the plant-mediated synthesis of mNPs. Microbial synthesis of mNPs is achieved extracellularly or intracellularly. In intracellular synthesis, metal ions are adsorbed by the microbial biomass or entered inside the microbial cell, where they are enzymatically reduced into mNPs. Extracellular biosynthesis of mNPs was investigated for the fabrication of gold and silver nanoparticles using fungal biomass (*Verticillium sp.*). At first, metal ions were adsorbed by the fungal mycelium; this adsorption was caused by the weak electrostatic forces between the positively charged metal ions and the negatively charged enzymes of the cell wall, where they were reduced into mNPs. Nicotinamide adenine dinucleotide (NADH) and NADH-dependent nitrate reductase are the vital enzymes that are responsible for the biosynthesis of mNPs [85]. Similarly, the NADPH-dependent mechanism of silver nanoparticle synthesis was studied in *Bacillus licheniformis* (Figure 2), this study established the role of nitrate reductase in metal ion reduction that led to the formation of silver nanoparticles [86].

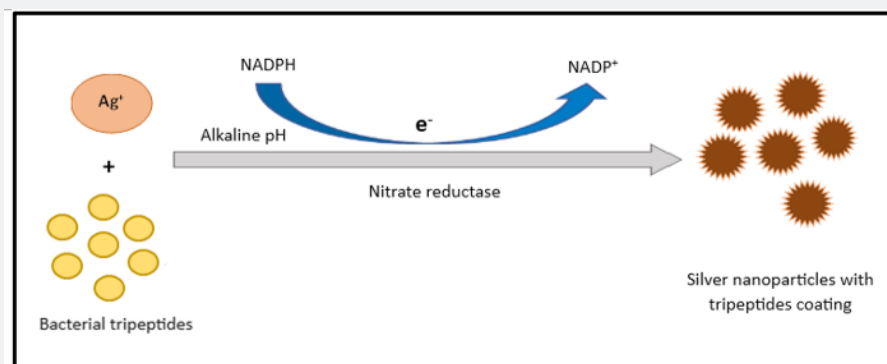
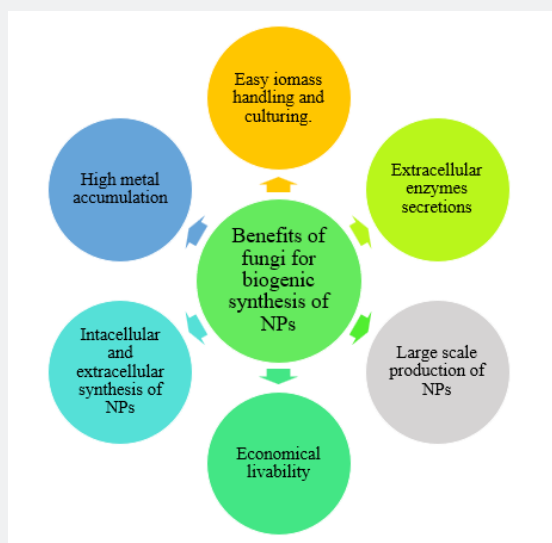


Figure 2: NADPH-dependent mechanism of silver nanoparticles synthesis.

### Fungal endophytes for the synthesis of metal nanoparticles

Endophytic fungi are a rich source of biologically active compounds, enzymes, and secondary metabolites (e.g., alkaloids, phenols, peptides, acids, quinones, terpenoids, steroids), which have diverse therapeutic potential. Several compounds of medicinal value have also been isolated and purified from different endophytic fungi. In 1993, *Taxomyces andreanae* was investigated to produce taxol, a new anticancer compound [87]. Similarly, antimicrobial and anti-inflammatory compounds such as clavate, pestacin, and isopestacin have also been reported from

endophytic fungi [88,89]. Chitinase, cellulase, and lipase are the most widespread enzymes that are produced by few endophytic fungi, and these enzymes have a main role in the management of phytopathogens [90]. Endophytic fungi have many advantages over the use of other microbes (Figure 3); hence these are considered attractive agents for the biosynthesis of mNPs. Psychosynthesis of nanoparticles is an innovative method that is being explored for the development of eco-friendly and safe nanomaterials [91]. While endophytic fungal species have been extensively studied for their potential to produce various biologically active compounds, there is limited literature on the biogenic fabrication of mNPs using extracts from these fungi.



**Figure 3:** Advantages of fungi as biological material for the synthesis of nanoparticles.

Metal nanoparticle (mNPs) synthesis can be achieved using fungi, the synthesis mechanism may be intracellular and extracellular. In the intracellular synthesis of nanoparticles, metal salt that is used as a precursor is mixed with the fungal biomass cultures that are absorbed by the mycelial biomass within a certain time. Subsequently, extraction of the intracellularly fabricated nanoparticles is carried out using different physicochemical treatments which help to disintegrate the fungal mycelium and facilitate the extraction of nanoparticles [92]. In the extracellular synthesis of nanoparticles, the fungal biomass filtrate is used as a reducing agent and the metal salt solution is added to this fungal biomass extract. After a certain time, metal ions are reduced into nanoparticles and the resultant nanoparticles can be easily separated from dispersion by centrifugation. This method is extensively employed for the biogenic synthesis of nanoparticles using fungi as there is no physicochemical method required for the release and extraction of nanoparticles [93]. However, to obtain the pure form of nanoparticles, different methods including filtration, dialysis, and ultracentrifugation can be used to remove the impurities [94].

### Metal nanoparticle synthesis using extracts from fungal endophytes

Metal nanoparticle synthesis using extracts from endophytic fungi is accomplished in a few generalized steps, although slight modifications can be made in the synthesis process depending on the mNPs. Fungal isolates are procured from a fungal culture bank, and cultured on potato dextrose agar slants at 28°C. These fungal slants are sub-cultured from time to time to maintain their viability. The sub-cultured fungal isolates are used to produce fungal biomass, broth media is used to produce fungal biomass in larger amounts. For this purpose, an incubator shaker is

used, where the cultures are incubated at 28°C for seven days, afterwards the biomass is harvested and thoroughly washed with the distilled water to remove the particles of growth media. The fungal biomass extraction is carried out using water as a solvent. For aqueous extraction, 25g of fresh fungal biomass is treated with 100mL of distilled water and boiled for up to 30min, boiling can cause the release of fungal metabolites into water by disintegrating the fungal biomass. The obtained fungal biomass extract is used for the synthesis of mNPs. Fungal biomass extract is gradually mixed with precursor salt solution and incubated at room temperature, which leads to the reduction of metal ions into mNPs. The synthesized mNPs are collected by centrifuging the nanoparticle solution at 6,000rpm, and obtained mNPs pellets are further washed with milli Q water to remove the contaminants. The obtained mNPs are analyzed through different characterization techniques, to confirm their size and structural morphology [95].

### Importance of biologically synthesized metal nanoparticles

The biosynthesis of mNPs has numerous advantages over the chemically synthesized mNPs. Biosynthesis of mNPs involves the use of biological materials which are renewable and biodegradable. This method is free from the use of toxic chemicals and reagents. It is a simple, rapid, cheaper, safer, and environmentally benign approach as it reduces environmental pollution by limiting the generation of harmful by-products [83]. The biological method is a time-saving approach that is accomplished in only a few steps, where higher yields of nanoparticles can be obtained in less time [96]. Bio-fabricated mNPs are biocompatible and safer for biomedical applications and these are effectively used for cancer treatment [97]. Biosynthesized mNPs are very selective in adsorption, only particular biological molecules that are

compatible with the mNPs are adsorbed on the nanoparticle surface and form a corona that mainly makes an interaction with the living cell. The formation of corona offers enhanced activity of mNPs as compared to simple nanoparticles. Therefore, biologically fabricated mNPs show excellent biological activities than the chemically fabricated mNPs [96].

### Common metal nanoparticles synthesized from extracts of fungal endophytes

The biogenic synthesis of mNPs using fungal endophytes is an emerging approach being explored for the creation of environmentally friendly and safe nanomaterials [91]. While the endophytic fungal community has been widely studied for the identification of various biologically active compounds, there is limited literature on the biogenic production of nanoparticles using extracts from these fungi (Table 3).

#### Silver nanoparticles (AgNPs)

Silver is a vital and most well-known metal that has a long therapeutic history from centuries ago. It is considered a most excellent, safe, inorganic antimicrobial agent, as it possesses an extremely high surface area that facilitates high contact with microbes. AgNPs are the most novel nanomaterials that have been investigated extensively and several of their applications also have been reported [98]. AgNPs seem to be very active, they have been observed as one of the most useful nanomaterials than the other mNPs, as they show pronounced antimicrobial activity against many harmful microbes [99]. The synthesis of safer and biologically potent nanoparticles is one of the main advantages of biological methods [100]. Endophytic fungi are a great source of many biologically active compounds, therefore biosynthesis of AgNPs using extracts from endophytes leads to the fabrication of highly effective nanoparticles.

AgNPs are the most widely studied nanoparticles, which have been fabricated using extracts from endophytic fungi in the past few years. Plant-mediated synthesis of AgNPs was reported using extracts of an endophytic fungus, *Pestalotiopsis microspore*, where the aqueous mycelial extracts were used as a bio-reducing agent. The extracellularly biosynthesized Ag-NPs were characterized through different techniques which revealed their size and structural morphology, Ag-NPs were found stable with a high zeta potential value (-35.7mV). Furthermore, their antioxidant and cytotoxic activity was also investigated against the human and mouse cancer cell lines, where they showed significant results [101]. Similarly, in another study, the biological fabrication of Ag-NPs was performed using a supernatant of the endophytic fungus *Alternaria sp.* Transmission electron microscopic micrographs (TEM), showed the globular shapes of nanoparticles with an average size of 30 nm. Moreover, these nanoparticles were also tested for their antimicrobial potential where they exhibited pronounced effects against human pathogens [102]. Most recently, a study reported the extracellular biosynthesis of AgNPs was

achieved using mycelial extracts of *Pestalotiopsis paucisetata* [103].

Fungus-mediated extracellular biosynthesis of Ag-NPs was investigated using extracts from endophytic fungi, (*Penicillium janthinellum*, *Aspergillus conscientis*, and *Phomopsis sp.*). These bio-fabricated nanoparticles were observed as poly-dispersed in nature, hexagonal and spherical shapes were seen with a size range of 6-16nm. The antibacterial potential of these nanoparticles was also investigated against 12 gram-negative and gram-positive bacterial strains using agar well diffusion assay, where AgNPs cause significant inhibition of the tested bacterial species [104]. Extracellular biosynthesis of AgNPs was also studied using *Penicillium italicum* extract, the synthesis was confirmed and analyzed through different characterization techniques, and transmission electron microscopic analysis showed the circular shapes with a size range from 14.5nm to 23.3nm. Antibacterial effects of resulted nanoparticles were also investigated against *Salmonella enterica*, *Bacillus cereus*, *Escherichia coli*, and *Staphylococcus aureus*, remarkable results were observed, and the maximum inhibition zone (20mm) were calculated for *S. enterica*. Additionally, the anticancer activity of biosynthesized AgNPs using MTT assay was also evaluated against the HEP-2 cancer cell line [105].

Biological synthesis of AgNPs using endophytic fungi was carried out, where the biomass extracts of *Fusarium oxysporum* were used as a bi-reducing agent for the formation of AgNPs. SEM micrographs of the resulting nanoparticles exhibit spherical-shaped nanoparticles with a size range from 19-50nm. AgNPs showed excellent bactericidal effects against five tested bacterial species [106]. The biosynthesis of AgNPs was achieved using extracts from *Penicillium* species isolated from *Glycosmis mautitiana*. During the synthesis process, fungal biomass extracts caused the reduction of silver ions into AgNPs. Bio-fabricated AgNPs were analyzed through different characterization techniques. The resulting nanoparticles were examined for their antibacterial and antioxidant potential. In addition, the anti-inflammatory activity of bio-fabricated AgNPs was also tested where they strongly reduced the production of tyrosine kinase, xanthine oxidase, and lipoxigenase [107].

#### Copper nanoparticles (CuNPs)

Copper is an essential elemental micronutrient of plants. It is one of the crucial parts of many vital compounds, which regulate plant growth and development [108]. It is relatively cheap metal that has substantial antimicrobial activity as compared to other known metals [109]. In the past few years, the use of mNPs in biology has been gaining much attention. These inorganic mNPs possess excellent biological activities, which make them the most suitable components in medicinal preparations [110]. Biosynthesis of CuNPs using extracts from endophytic fungal isolates is considered the most effective way to fabricate nanoparticles with desired properties and outstanding biological potential. Recently,

the plant-mediated synthesis of CuNPs using fungal extracts has been reported by a few researchers.

Fungus-mediated synthesis of CuNPs was documented with *Stereum hirsutum*, using three metal salts as precursors [CuSO<sub>4</sub>, CuCl<sub>2</sub>, and Cu (NO<sub>3</sub>)<sub>2</sub>] under different pH conditions. A higher yield of nanoparticles was observed under basic conditions with 5mM CuCl<sub>2</sub>. FTIR spectrum revealed the amine groups as the main capping agent of nanoparticles, which verified the biosynthesis of nanoparticles due to the extracellular proteins. Resulted nanoparticles were confirmed through different characterization techniques, where TEM images showed that the synthesized CuNPs were globular in shape with a diameter range from 5-20nm [111]. In a recent study, the bio-fabrication of CuNPs was reported using the extract from *Trichoderma asperellum*.

FTIR spectra of biologically synthesized nanoparticles revealed the presence of amide and aromatic groups of secondary compounds of biomass extracts as reducing and capping agents of CuNPs. The average size was measured as 110nm using a particle size analyzer. Whereas the cubic crystalline structure of Cu-NPs was observed with XRD [112]. Biosynthesis of CuNPs using fungi has been carried out by only a few researchers, therefore there is only a little literature available on fungus-mediated green synthesis of nanoparticles. Pavani, Kumar, and Sangameswaran [113], studied the biogenic synthesis of lead nanoparticles using extracts from *Aspergillus* species. Similarly, some of the fungal species (*Penicillium vaksmanii*, *Penicillium citrinum*, and *Penicillium aurantiogriseum*) that were isolated from soil have also been studied for the synthesis of copper nanoparticles [109].

### Gold nanoparticles (AuNPs)

Gold is an expensive novel metal, that has been traditionally used as a therapeutic element. AuNPs are considered one of the most compatible and safer nanoparticles for biomedical applications like cancer treatment. The size of AuNPs can be controlled during the synthesis process, where the surface modifications are done using different functional groups [114]. AuNPs can be synthesized in different structural forms including nano shells, nanospheres, nanorods, and nanocages [115]. Their typical and exceptional characteristics such as small size, larger surface area to volume ratio, versatile nature, and non-toxicity make them very appropriate for different applications [116].

AuNPs are other extensively reported nanoparticles, which have been synthesized using extracts from entophytic fungi. Biological synthesis of AuNPs using the extract from an endophytic fungus (*Cladosporium cladosporioides*) was achieved. The synthesis of bio-fabricated AuNPs was confirmed and analyzed using valid techniques like UV-vis spectrophotometer, Fourier transform infrared spectroscopy, field emission scanning electron microscopy, energy dispersive x-ray spectroscopic, dynamic light scattering, atomic force microscopy and x-ray diffraction. FTIR analysis revealed the reduction of the gold ions to AuNPs, NADPH-

dependent reductase, and phenolic compounds of fungal extract were found to be responsible for this reduction. Antimicrobial and free ion radical scavenging activities of biosynthesized AuNPs were also assessed where significant results were observed [117].

Recently, the synthesis of AuNPs was reported using *Fusarium solani*. Fungal biomass extract acted as a reducing and stabilizing agent during the extracellular synthesis of AuNPs. The synthesis of the nanoparticles was investigated using different techniques, and FTIR results authenticated the role of proteins in the fabrication of nanoparticles. The average size of Au-NPs was observed as 45 nm which was analyzed using SEM analysis. Cytotoxicity effects of green synthesized AuNPs were also tested against cervical cancer cell lines and human breast cancer, where these nanoparticles showed dose-dependent cytotoxic effects [118]. Biogenic formation of AuNPs using an endophytic fungal isolate was established, and fungus extract was used as a bio-reducing agent (enzymes) that led to the formation of AuNPs. Results showed the fabrication of spherical-shaped AuNPs that were mono-dispersed in nature [119].

### Zinc oxide nanoparticles (ZnONPs)

Zinc is another vital metal of the earth's crust, that exists in various forms of oxides [120]. The Biosynthesis of ZnNPs is receiving much attention as it has several valuable applications in different areas of science. The biological synthesis of ZnONPs using fungal extracts is less investigated, therefore there are only a few pieces of literature available. In a recently reported study, extracellular fabrication of ZnONPs was studied using mycelial extracts from *Aspergillus niger*. The fungal extract-mediated ZnONPs were analyzed using valid characterization techniques, and FTIR analysis established the involvement of carboxylic acid in the reduction of zinc ions to ZnONPs. Resulted ZnONPs were also proved effective for their dye removal [121]. The extracellular biosynthesis of ZnONPs was reported using an extract of *Aspergillus fumigatus*, where zinc nitrate was used as precursor metal salt. Furthermore, the effects of bio-fabricated ZnONPs on *Cyamopsis tetragonoloba* were also studied to observe the role of ZnNPs in enzyme secretion in *C. tetragonoloba* [122].

### Iron nanoparticles (FeNPs)

Iron is one of the most abundant and common metals on the earth, though it has been unnoticed due to the occurrence of its oxides and other metals. However, FeNPs have received much attention due to their wide range of applications in various fields of science and technology [123]. Nowadays, the present challenge is the fabrication of safe, environmentally friendly, and economical nanomaterials. Plant-mediated synthesis of FeNPs using extracts from endophytic fungi is a reliable method as it does not include the use of noxious chemicals and reagents, high temperature, and pressure requirements. Biosynthesis of FeONPs was reported using two fungal species, (*Fusarium oxysporum* and *Verticillium sp.*). Fungal extracts were treated with ferrous and ferric salts,



where these metal ions were reduced by the extracellular secreted fungal proteins leading to the synthesis of FeONPs at room temperature. Further characterization of resulted nanoparticles revealed the synthesis of crystalline magnetite nanoparticles [124-141].

### Factors influencing nanoparticle synthesis from extracts of fungal endophytes

Several process factors greatly affect the shape, size, stability, and unique physicochemical properties of nanoparticles during the synthesis process. Therefore, the developmental design of the synthesis process and optimization of the growth parameters is the main key that substantially controls the physicochemical characteristics of nanoparticles [142]. Factors affecting the synthesis of mNPs include pH, temperature, pressure, shape and size, and incubation time. Different methods are used for the synthesis of mNPs, such as physical, chemical, and biological methods. Each method has its advantages while some methods have certain limitations. The use of hazardous chemicals, reagents, and other extreme conditions in conventional physicochemical methods limits their application. Nonetheless, the biological approach for mNPs fabrication is considered a simple, safe, and environmentally friendly approach [143]. Among all factors, pH is one of the foremost factors that affect the biosynthesis of mNPs during the growth process. It has been documented by several researchers that the pH of the reaction mixture greatly influences the mNPs synthesis and has a major role in defining the shape and size of mNPs. Hence, by changing the pH of the reaction mixture, the size and shape of the mNPs can be controlled [144].

The other main factor is the temperature which influences the nanoparticle synthesis. High temperature is required in chemical and physical methods, a temperature < 350°C is required for the chemical method, while the physical method requires an extreme temperature of more than 350°C. On the other side, biogenic fabrication of mNPs using biological materials can be achieved at room temperature, or in some cases a temperature less than 100°C is required for synthesis [145]. Pressure is also considered a dominant factor in physicochemical methods, that affects the mNPs shape and size during the synthesis process. Physical and chemical methods require high pressure, whereas biosynthesis is performed at ambient pressure [146]. Incubation time or interaction time (time for which reaction mixture is kept) has also a key role as it defines the quality of synthesized mNPs during the biological synthesis. Storage and light conditions also affect the nanoparticles' characteristics, and hence the physicochemical properties of mNPs are changed over a while. Incubation of mNPs reaction mixture for a long time may cause the synthesis of larger-sized mNPs and aggregation of mNPs that significantly influence their biological activity [147].

### Applications of myco-synthesized metal nanoparticles

In recent years, antimicrobial resistance to antibiotics has

risen, and many commercial organic antimicrobial drugs can be harmful to humans and animals, potentially causing severe allergic reactions. To address this challenge, inorganic materials present a promising solution due to their safety, high reactivity, and stability under extreme conditions. Metal nanoparticles have been extensively studied for their antimicrobial properties against various pathogens.

The antimicrobial effectiveness of mNPs is influenced by their size and the materials used for their synthesis. Currently, nanotechnology provides a robust framework for developing highly active antimicrobial agents. Key mNPs, such as silver, zinc oxide, titanium oxide, iron, gold, and magnesium oxide, exhibit broad-spectrum activity against diverse microorganisms [148]. Recent studies have highlighted the bactericidal effects of these nanoparticles against various bacterial species. Their antimicrobial potential is closely linked to their small size and large surface area-to-volume ratio, which enhance interaction with microbial cell membranes [149,150], while essential metal ions are crucial for microbial biochemical processes, lower concentrations can damage cell organelles and DNA, and higher concentrations can be toxic. The diverse applications of mNPs stem from their antimicrobial properties [151,152].

AgNPs stand out as exceptional antimicrobial agents, demonstrating broad-spectrum efficacy against pathogenic bacteria such as *Acinetobacter baumannii*, *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus epidermidis*, *Salmonella typhi*, *Micrococcus luteus*, *Enterococcus faecium*, *Listeria monocytogenes*, and *Staphylococcus aureus* [153,154]. These nanoparticles find applications in biomedicine, agriculture, wastewater treatment, food packaging, metal removal, and the textile industry [155]. AuNPs are another well-known antimicrobial agent that is safer for human cells, making them suitable for various biomedical applications due to their excellent biocompatibility [148]. Modifications to their physicochemical properties, such as the incorporation of photosensitizers, enhance their reactivity, allowing them to kill multidrug-resistant bacteria and inhibit cancer cell proliferation [156]. Antibiotic-embedded AuNPs (e.g., *vancomycin*, *ampicillin*, *cefactor*) have also been studied for their bactericidal effects against both Gram-positive and Gram-negative bacteria [157].

CuO-NPs have also been reported for their antimicrobial activity against various microbes. The exact mechanisms behind their antibacterial action require further investigation. Some studies suggest that copper ions damage cell membranes and generate reactive oxygen species, leading to oxidative stress and ultimately microbial death. Research on CuNPs has demonstrated their antibacterial activity against *Bacillus subtilis* and *Bacillus anthracis*, with the high affinity of CuNPs for carboxyl and amine groups on bacterial cell surfaces contributing to their bactericidal effects [151].

## Challenges and remedial strategies

Nanotechnology is an interdisciplinary field that brings together experts to design and develop materials with significant biomedical potential. Microbial nanotechnology, which utilizes microbes for nanoparticle synthesis, has gained attention as a simple, safe, economical, and eco-friendly method [158]. Metal nanoparticles have crucial therapeutic applications in biology and medicine, highlighting the need for biocompatible and safe nanoparticle development [159]. Researchers worldwide are exploring microbial-mediated synthesis due to the ability of microorganisms to bio-reduce metal ions [160]. A key challenge in this process is selecting the most suitable microorganism based on properties like pathogenicity and biological activity. Achieving controlled size and uniformity in nanoparticles is essential for their effectiveness. This can be accomplished by optimizing growth conditions, including the concentration of reducing agents and precursor metal salts, temperature, pH, and incubation time.

Microbes act as bio factories that produce diverse metabolites, making the identification of effective bio-reducing agents another important challenge. Culturing microbes under optimal conditions is crucial for maximizing the production of enzymes that facilitate nanoparticle synthesis. Despite the advantages of biological methods, challenges remain in scaling up production due to lower yields compared to chemical methods, which can produce nanoparticles at twice the yield. Additionally, laboratory-scale synthesis methods differ from industrial applications. However, biological synthesis is cost-effective, as it eliminates the need for chemical reagents, organic solvents, and high temperatures [161], making it a promising approach for nanoparticle production.

## Prospects

The biogenic fabrication of mNPs using fungal extracts is an innovative aspect of myco-nanobiotechnology, though it currently faces several challenges related to nanoparticle development, such as size, shape, structural morphology, crystalline structure, agglomeration, and stability. Despite these issues, biogenic synthesis is considered environmentally friendly and warrants further investigation, particularly into the mechanisms and proteins involved in the synthesis process. Downstream processing, which involves the separation and purification of nanoparticles from contaminants, remains relatively unexplored. Physical methods like heating, centrifugation, ultrasound, freeze-thawing, and osmotic shock are typically used, with a preference for minimizing chemical methods to ensure the safety of the nanoparticles. While biogenic synthesis is often conducted at a small scale, optimizing production processes and selecting appropriate microorganisms can facilitate commercial-scale synthesis. This optimization is crucial for developing a biologically safe and sustainable system for nanoparticle production. Additionally, cost-effectiveness must be evaluated in comparison to conventional methods. Recent advancements suggest that applying the twelve principles of green chemistry could enhance the sustainability of biogenic nanoparticle fabrication, representing a significant step towards

a greener future.

## Conclusion

Nanotechnology involves the synthesis, manipulation, and application of materials at the nanoscale, typically ranging from 1 to 100nm. Traditionally, nanoparticles have been synthesized using a variety of physical and chemical techniques; however, the use of hazardous chemicals often limits their application. An attractive alternative is the biological approach, which helps mitigate toxicity concerns. Biogenic synthesis of mNPs utilizes various biological materials, including plants and microbes, with fungal endophytes emerging as a reliable source. Fungal extracts serve as both reducing and capping agents, facilitating the conversion of metal ions into nanoparticles. Research has demonstrated the effectiveness of fungal extracts not only for nanoparticle synthesis but also for their antimicrobial properties against pathogenic microbes. The characteristics of nanoparticles are significantly influenced by growth factors, and optimizing reaction conditions can yield nanoparticles with desired properties. Biogenic synthesis is relatively simple, clean, sustainable, and cost-effective, aligning well with the principles of green chemistry. Future studies should further explore the potential of fungal endophytes in the synthesis and application of mNPs.

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